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Indian Standard

IS 11479 (Part 1) : 2021

जीवाणुरोधी प्रसाधन साबुन — विशिष्टि

भाग 1 ठोस बट्टी

(दूसरा पुनरीक्षण)

Antibacterial Toilet Soap — Specification

Part 1 Solid Cake

(Second Revision)

ICS 71.100.40

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FOREWORD

This Indian Standard (Part 1) (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Soaps and Other Surface Active Agents Sectional Committee had been approved by the Chemical Division Council.

Human skin provides a favorable environment for the existence and multiplication of a variety of microbes. The conventional toilet soap washes away the germs but does not kill them. The function of an antibacterial or antiseptic toilet soap is not only to clean the skin, but also to reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of sweat by bacteria.

The antibacterial toilet soap is especially effective against staphylococcus and similar bacteria which have the habit of residing in the under layers of skin. The antibacterial are substantive to the skin and this tackles the microbes between two washes. Antibacterial toilet soaps shall be used regularly to be effective.

IS 11479 'Antibacterial toilet soap' was first published in 1985 and subsequently revised in 2001 by splitting standard in two parts. Part 1 covered Solid cake and Part 2 covered Liquid. This standard supersedes IS 11479 (Part 1) : 2001 'Antibacterial toilet soap — Specification: Part 1 Solid Cake. During the last revision in 2001, it was decided to eliminate use of hexachloroprene as antibacterial agent. Trichlorocarbanilide (TCC) on heating decomposes to chloroanilines which can be harmful to skin and hence the limit and method for determination of chloroaniline was also incorporated in the last revision.

Antibacterial toilet soaps are not general consumer cosmetic products as they contain Triclosan (TCN) and Trichlorocarbanilide (TCC) as antibacterial agent which could cause harmful side effects. Hence, it is recommended that compliance of advisory in Drug and Cosmetic Rules concerning to user's safety need to be followed for manufacturing and selling of product.

In this revision, the limit of Triclosan (TCN) and Trichlorocarbanilide (TCC) has been aligned with limits prescribed in IS 4707 (Part 2) Classification for cosmetic raw materials and adjuncts: Part 2 List of raw materials generally not recognized as safe for use in cosmetics.

A scheme for labelling environment friendly products to be known as ECO Mark has been introduced at the instance of Ministry of environment, Forest and Climate Change (MoEF&CC). The ECO Mark shall be administered by the Bureau of Indian Standards (BIS) under the *BIS Act,* 2016 as per the Resolution No 71 dated 20 February 1991 published in the Gazette of the Government of India. For a product to be eligible for ECO Mark, it shall carry the standard mark of BIS for quality besides meeting additional optional environment friendly (EF) requirements.

The requirements of the conventional grade of toilet soaps are given in IS 2888 'Toilet soap'.

There is no ISO specification on this subject. This standard is formulated based on indigenous technology and data available.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 1 SOLID CAKE

(Second Revision)

1 SCOPE

This standard (Part 1) prescribes the requirements, methods of sampling and test for antibacterial toilet soap, solid cake.

2 REFERENCES

The Indian Standards listed below contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No	*Title*
286 : 2018	Methods of sampling and test for soaps
1070 : 1992	Reagent grade water
4707 (Part 1) : 2020	Classification for cosmetics raw materials and adjuncts: Part 1 Colours
4707 (Part 2) : 2017	Classification for cosmetic raw materials and adjuncts: Part 2 List of raw materials generally not recognized as safe for use in cosmetics
4955 : 2020	Household laundry detergent powders
7597 : 2001	Surface activity agents — Glossary of terms
11601 : 2002	Methods of safety evaluation of synthetic detergents — Tests for skin irritation and sensitization potential of synthetic detergents
13424 : 2001	Safety evaluation of bathing bars and toilet soaps — Method of test

3 TERMINOLOGY

For the purpose of this standard, the definitions given in **3** of IS 286 and IS 7597 shall apply.

4 REQUIREMENTS

4.1 Description

Antibacterial toilet soap (solid cake) shall be a high grade, thoroughly saponified, milled soap or homogenized soap or both, white or coloured, perfumed, and compressed in the form of firm and smooth cakes, and shall possess good cleaning and lathering properties.

4.2 Ingredients

4.2.1 In addition to perfume, moisture, normal colouring matters, preservatives acceptable in toilet soaps in general, the antibacterial soap shall contain permitted antibacterial agent (*see* **4.2.2**). The label shall clearly state the antibacterial agent used and its level. The soap shall pass the antibacterial activity test when determined by the method given in Annex A.

4.2.2 Triclosan (TCN) and trichlorocarbanilide (TCC) shall not exceed the limits prescribed in IS 4707 (Part 2) 'Classification for cosmetic raw materials and adjuncts: Part 2 List of raw materials generally not recognized as safe for use in cosmetics by mass either singly or in combination, when tested by the method prescribed in Annex B.

4.2.3 Chloroaniline content shall not exceed 10 ppm when tested by the method prescribed in Annex C.

NOTE — TCC is not heat stable and decomposes into chloroanilines on prolonged heating above 60 °C. If TCC is used in soap, the manufacturer should take care that such soap is not subjected to temperature above 60 °C during the entire manufacturing process or during storage.

4.2.4 The material may contain permitted colour as given in IS 4707 (Part 1), preservatives, medicaments and such additional substances as are declared on the label. The material shall not contain any ingredient above the limit as given in IS 4707 (Part 2). The material shall pass the test for skin irritant and sensitization potential when evaluated as per the method prescribed in IS 11601. The materials shall also be non-injurious to skin when tested by the methods prescribed in IS 13424.

4.3 Antibacterial toilet soap, solid cake shall also comply with the requirements specified in Table 1.

Table 1 Requirements for Antibacterial Toilet Soap, Solid Cake

(*Clause* 4.3)

Sl No.	Characteristic	Requirement	Method of Test (Ref to Cl no. in IS 286)
(1)	(2)	(3)	(4)
i)	Total fatty matter, percent by mass, *Min*	76	16
ii)	Rosin acids[1)], percent by mass of total fatty matter, *Max*	3	15
iii)	Free caustic alkali, as sodium hydroxide (NaOH), percent by mass, *Max*	0.05	7
iv)	Free carbonated alkali, as sodium carbonate (Na_2CO_3), percent by mass, *Max*	1	29
v)	Matter insoluble in alcohol), percent by mass, *Max*	2.5	6

[1)] If rosin is not used as an ingredient during the manufacture of soap there is no need to test the requirement of rosin acid content.

4.3.1 *Calculation of Results*

Antibacterial toilet soap is liable to lose moisture on keeping. The results of analysis in respect of free caustic alkali, free carbonated alkali and matter insoluble in alcohol shall be recalculated in relation to the minimum specified total fatty matter by means of the following equation:

$$\text{Recalculated result} = \text{Actual result} \times \frac{\text{Minimum specified total fatty matter}}{\text{Actual total fatty matter}}$$

4.4 Additional Requirements for ECO-Mark

4.4.1 *General Requirements*

4.4.1.1 The product shall conform to the requirements for quality, safety and performance prescribed under **4.1** to **4.3.**

4.4.1.2 The manufacturer shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the provisions of *Water* (*Prevention and Control of Pollution*) *Act,* 1974 and *Air* (*Prevention and Control of Pollution*) *Act,* 1981 along with the authorization, if required, under the *Environment* (*Protection*) *Act,* 1986 while applying for ECO- Mark.

4.4.2 *Specific Requirements*

4.4.2.1 The antibacterial toilet soap shall neither contain any synthetic detergent when tested as per the method given in Annex B and C of IS 4955 nor any phosphate when tested as per the method prescribed in **21** of IS 286.

4.4.2.2 The antibacterial toilet soap shall pass the test for dermatological safety when evaluated as per the method prescribed in IS 13424.

5 PACKING AND MARKING

5.1 Packing

The product shall be packed as agreed to between the purchaser and the supplier.

5.1.1 For ECO-mark the product shall be packed in such packages which are made from recyclable/reusable or biodegradable material and declared by the manufacturer and may be accompanied with detailed instructions for proper use.

5.2 Marking

The packages shall be securely closed and marked with the following particulars:

a) Name and address of manufacturer;
b) Brand name of the material and recognized trade mark, if any;
c) Net mass when packed;
d) Batch No. or lot No. in code or otherwise;
e) Month and year of manufacture; and
f) The following identified critical ingredients in descending order of quantity; percent by mass.
   1) Total fatty matter (TFM),
   2) Matter insoluble in alcohol, and
   3) Antibacterial agent.

5.2.1 *Additional Information for Eco-Mark*

The criteria for which the product has been labelled as ECO-Mark.

5.2.2 *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

6 SAMPLING

6.1 For this purpose general precautions, scale of sampling and preparation of test samples shall be as prescribed in **4** of IS 286.

6.2 Number of Tests

6.2.1 Tests for determination of total fatty matter and free caustic alkali and matter insoluble in alcohol shall be conducted on each of the individual samples separately.

6.2.2 Tests for determination of all the remaining characteristics shall be conducted on the composite sample.

6.3 Criteria for Conformity

6.3.1 For each of the characteristics which has been determined on the individual samples (**6.2.1**) the mean (X) and the range (R) of the test results shall be calculated as follows:

$$\text{Mean } (X) = \frac{\text{the sum of test results results}}{\text{number of test results}}$$

Range (R) = The difference between the maximum and the minimum value of test results

The lot shall be deemed as conforming to the requirements given in **6.2.1** if the expression (X— 0.6 R) *is* greater than or equal to minimum value given in Table 1 and (X+ 0.6 R) is less than or equal to maximum value given in Table 1.

6.3.2 For declaring the conformity of a lot to the requirements of other characteristics determined on the composite sample, the test results for each of the characteristics shall satisfy the relevant requirement.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results or analysis.

ANNEX A

(*Clause* 4.2.1)

DETERMINATION OF ANTIBACTERIAL ACTIVITY

A-1 GENERAL

Two methods have been prescribed, namely, serial dilution method and substantively test. The serial dilution test shall be the screening test and the substantively test shall be the absolute test.

A-2 SERIAL DILUTION TEST

A-2.1 Outline of the Method

Antibacterial activity is determined by serial dilution method by comparing the effectiveness of antibacterial chemicals present in 10 micrograms of soap per milliliter specified as the maximum inhibitory concentration.

A-2.2 Apparatus

A-2.2.1 *Culture Tube, Rimless*, 150 mm ×18 mm.

A-2.2.2 *Sterilized Pipettes*, 10 ml, 5 ml and 1ml capacities.

A-2.2.3 *Loop Made of Stainless Steel or Platinum Wire*

A-2.2.4 *Conical Flasks*, 250 ml capacity.

A-2.3 Nutrient Broth

A-2.3.1 Dissolve 5 g of beef extract, 5 g of sodium chloride, 10 g of peptone in one litre of distilled water by warming over a water bath. Alternatively, commercial dehydrated media having constituent as indicated above may also be used. The manufacturer's instructions shall be followed for preparation of broth. Cool and adjust the *p*H to 7.2 to 7.6 with sodium hydroxide solution. Distribute 9 ml each to the culture tubes. Plug the tube with non-absorbent cotton wool and sterilize in an autoclave for half an hour at 1 kg/cm^2 pressure.

A-2.3.2 Take 99 ml and 90 ml of distilled water in two different 250 ml conical flasks. Plug them with non-absorbent cotton wool and sterilize in an autoclave.

A-2.3.3 Get a pure stain of *Staphylococcus aureus.* ATCC 6538 P, maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. Use a 24 h nutrient broth culture for the experiment.

A-2.4 Procedure

A-2.4.1 Aseptically transfer 1 g of the soap sample to the flask containing 99 ml of water. Dissolve by slight warming not exceeding 60 °C. Transfer 10 ml of this solution to another flask containing 90 ml of water. Take 1ml of this solution and add 9 ml of nutrient broth in a culture tube. This gives a concentration of 100 µg/ml.

A-2.4.2 To three tubes containing 9 ml nutrient broth add 1 ml of the above solution to each tube to get a concentration of 10 µg of soap per ml of nutrient broth in each tube. Inoculate the tubes with a loopful of the 24 h culture of *Staphylococcus aureus* and keep them in an incubator maintained at 37 ± 2 °C. Keep a control tube of nutrient broth containing the same concentration of soap (with or without culture).

A-2.4.3 If after 24 h incubation period, the liquid in all the three tubes is as clear as the control, the soap sample passes the test. Any turbidity more than the control shows the growth of bacteria.

A-3 SUBSTANTIVITY TEST

A-3.1 Basic Principles

For a soap to have antibacterial activity, it shall satisfy two criteria:

a) It shall show, antibacterial activity on the skin even after the soap is rinsed away, that is, the germicide should be retained on the skin under the conditions of use; and

b) The antibacterial activity should be retained on the skin for some period so as to provide protection to the skin.

A-3.2 The test devised gives a measure of both these properties. The test involves application of soap solution on the forearm, rinsing it off in running water and allowing it to dry. A mixed culture of skin flora isolated from 5 individuals (*see* **A-3.3.1**) is applied immediately in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors in 10 min is determined. Similarly the soap solution after rinsing is allowed to remain on the skin for 2 h. The test micro-organisms are applied to the skin at this time in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors is determined. If the reduction in survivors at this time is greater than 45 percent, the germicide is said to be substantive.

A-3.3 Method

A-3.3.1 *Test Micro-Organisms*

The test organisms, consist of a mixed skin flora, prepared by collecting washings from the arms and forearms of at least 5 individuals using 50 ml of sterile water in each case. Ten ml aliquot of each washing is individually inoculated into flasks containing

90 ml of sterilized nutrient broth. Culture is allowed to grow overnight at 30°C and flask showing turbidity are pooled together. The mixed culture is transferred through broth and grown as above at least 3 times and finally maintained on Tryptone-Agar-Glucose Yeast Extract (TGYE) agar, Trypticase Soy Agar (TSA), Nutrient Agar (NA) or similar agar slants. For a test culture, an overnight slant culture is suspended into sterile saline and adjusted to a cell population of 1×10^7 cells per ml.

A-3.3.2 *Test Procedure*

A-3.3.2.1 A number of 4 cm^2 areas (2 cm × 2 cm) are marked out on the inner side of the forearm. 0.1 ml aliquot of an 8 percent soap solution with germicide is applied onto individual squares and allowed to dry for 1 min. The areas are then washed with a gentle flow of tap water for two min, dried by blowing warm air. The retentivity of the germicide on skin and its antibacterial action are then assayed by applying 0.1 ml of mixed skin flora (10^7 cells/ml) onto 4 such squares at 0 h. Two of the squares are swabbed immediately using standard sterile cotton swab on a stick. Swabs are placed in 5 ml saline solutions.

Contents are shaken well in a vortex mixer and tenfold dilutions are prepared. Bacterial cells are assayed on TGYE agar, TSA or NA plates to determine the initial count. After 10 min, two other squares are swabbed and assayed in a similar manner.

A-3.3.2.2 In another set of tests, soap solutions are applied to the 4 more squares, rinsed and dried. After allowing 2 h interval, 0.1 ml of culture is applied as above to 4 squares. Two of the squares are swabbed and assayed at 0 h and remaining two after 10 min. Survivals at 0 h and after 2 h are determined.

A-3.4 The soap shall be considered to have passed the test if the percent kill is greater than or equal to 45 percent after two hours challenge.

ANNEX B

(*Clause* 4.2.2)

DETERMINATION OF TCC AND TCN IN SOAPS BY HPLC

B-1 PRINCIPLE

TCC and TCN are antibacterial agents, which are separated from other components in soap by normal phase or reverse phase liquid chromatography, detected spectrophotometrically and quantified by comparison with standard TCC and TCN. The method can estimate as low as 1 ppm of the above compounds.

Procedures for both normal and reverse HPLC has been described and provide the option to use either method whichever is available to the users. Both methods are comparable.

B-2 NORMAL PHASE HPLC

B-2.1 Reagents

B-2.1.1 *Iso-octane* — HPLC grade.

B-2.1.2 *Iso-propanol (2-propanol)* — HPLC grade.

B-2.1.3 *Hexane* — HPLC grade.

B-2.1.4 *Standard TCC* — 99 percent pure.

B-2. 1.5 *Standard TCN* — 99 percent pure.

B-2.2 Apparatus

B-2.2.1 *High Performance Liquid Chromatography*

Consisting of a pump, a sample injector of fixed volume with UV detector having variable wavelengths and a recorder.

B-2.2.2 *Standard Volumetric Flasks*

B-2.2.3 *Pipettes*

B-2.2.4 *Magnetic Stirrer*

B-2.2.5 *Millipore Filter Apparatus with 0.5 µ Filter*

B-2.2.6 *Column*

B-2.2.6.1 *Silica column*

Stainless steel 25 cm × 0.46 cm packed with Normal phase – Silica 5 µ (Lichrosorb Si-60)

B-2.2.6.2 *Cyano column*

Stainless steel 25 cm × 0.40 cm packed with (Lichrospher 100) cyano 5 µ.

NOTE — Either of the above columns (**B-2.2.6.1**/**B-2.2.6.2**) can be used depending on the availability.

B-2.2.7 *Mobile Phase*

B-2.2.7.1 *For silica column*

Transfer 20 ml of *iso*-propanol into a 500-ml volumetric flask and make up to mark with *iso*-octane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B-2.2.7.2 *For cyano column*

Transfer 50 ml of HPLC grade *iso*-propanol (2 propanol) into a 500 ml volumetric flask, fill up to the mark with hexane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B-2.2.8 *HPLC Conditions*

Detector wavelength	:	280 nm
Flow rate	:	0.5 ml/min
Injection volume	:	20 µl

Retention Time

Silica Column

TCN – 7.5 min

TCC – 19.2 min

Cyano Column

TCN – 4.0 min

TCC – 7.5 min

B-2.3 Procedure

B-2.3.1 *Standard Preparation* (*see* Note *under* **B-3.4**)

Weigh accurately 25 mg of triclosan (TCN) and 25 mg of TCC into a 100-m1 volumetric flask and make up to volume with the mobile phase and mix well. Pipette 1.0 ml of this solution in a 50 ml volumetric flask and dilute with mobile phase. Final concentration of TCC and TCN is 250 µg/50 ml (5.0 ppm).

B-2.3.2 *Sample Preparation*

Weigh accurately 1 g of homogenized sample into a 100 m1 standard flask, and dilute to the mark with mobile phase. Pipette 10 ml of the supernatant liquid to a 50 m1 volumetric flask, dilute with mobile phase, to the mark, and filter through 0.45 µm filter.

B-2.3.3 *Chromatography*

Equilibrate the column, maintained at a temperature of 30 °C, with the mobile phase having flow rate of 0.5 ml/min for *iso*-octane – *iso*-propanol mobile phase and 1.0 ml/min for Hexane – *iso*-propanol mobile phase for 30 min. Set the wave length at 280 nm. Inject 20 µl of standard solution and then sample solutions. Measure area of the peaks of respective retention time for standard and sample.

B-2.4 Calculation

$$\text{TCN, percent by mass} = \frac{\text{Area of sample for TCN} \times \text{Concentration of standard TCN} \times 100}{\text{Area of standard TCN} \times \text{Concentration of sample}}$$

$$\text{TCC, percent by mass} = \frac{\text{Area of sample for TCC} \times \text{Concentration of standard TCC} \times 100}{\text{Area of standard TCC} \times \text{Concentration of sample}}$$

B-3 REVERSE PHASE

B-3.1 Reagents

B-3.1.1 *Methanol* — HPLC grade.

B-3.1.2 *Sodium Dihydrogen Phosphate Monohydrate —GR grade.*

B-3.1.3 *Standard TCC*

B-3.1.4 *Standard TCN (TCS)*

B-3.2 Apparatus

B-3.2.1 *Column*

Octyldimethylsilyl (C-DB)

Supercosil LC-8-DB 15 cm × 4.6 mm particle size 5 µm

B-3.2.2 *Mobile Phase*

MeOH/0.01 M Phosphate buffer 62 : 38 *v/v*

0.01 M Phosphate buffer : Dissolve 1.38 g NaH_2PO_4. H_2O in 800 ml water and adjust *p*H to 3.0 by adding 10 percent phosphoric acid solution and makeup the volume to 1 000 ml with water.

B-3.3 Procedure

B-3.3.1 *Standard Preparation* (*see* Note *under* **B-3.4**)

B-3.3.1.1 Weigh accurately about 90 mg of TCN. Dissolve in methanol and make up to 1 000 ml volumetric flask with methanol.

B-3.3.1.2 Weigh about 110 mg of TCC, dissolve well with methanol, and make up the volume to 1 000 ml.

B-3.3.1.3 Accurately pipette out 10 ml of solution prepared in **B-3.3.1.1** in to the volumetric flask of 100 ml containing 10 ml solution of TCC (*see* **B-3.3.1.2**). Makeup the volume with methanol. Then accurately pipette 5 ml of the solution into a 50 m1 volumetric flask. Make up to the volume with methanol. Filter this standard solution through 0.45 µm filter.

B-3.3.2 *Sample Preparation*

Weigh accurately about 1.0 g of product, dissolve in methanol and make up to 100 ml in a volumetric flask with methanol. Filter this sample solution through 0.45 µm filter.

B-3.3.3 *HPLC Conditions*

Detector wavelength : 280 nm

Column temperature : 35 °C

Flow rate : 1.0 ml/min

Injection volume : 10 µl

Prepare the standard solution and the sample solution at the same time. Inject the standard solution three times and calculate the average of peak count/area for each ingredients (TCC and TCN). Inject 10 µl of the sample solution and determine each ingredients percentage by the calculation shown as per **B-3.4**.

B-3.4 Calculations

$$\text{TCN, percent by mass} = \frac{M_S \times A_r \times F}{A_S \times M_t \times 100}$$

$$\text{TCC, percent by mass} = \frac{M_S \times A_r \times F}{A_S \times M_t \times 10}$$

where

M_S = mass of the standard (g);

A_S = Averaged peak area of the standard;

M_t = mass of the test sample (g);

A_r = Peak area of the test sample; and

F = Purity of standard (percent).

NOTE — Both TCC and TCN are photosensitive, hence standards should be freshly prepared.

ANNEX C

(*Clause* 4.2.3)

DETERMINATION OF CHLOROANILINE

C-1 PRINCIPLE

The chloroanilines are extracted from soap with dimethyl sulfoxide and diazotized with nitrous acid. The reaction products are then coupled with N-1-(naphthyl) ethylenediamine hydrochloride to produce coloured compounds which are estimated spectrophotometrically.

C-2 SAFETY PRECAUTIONS

Dimethyl sulfoxide (DMSO) is readily absorbed into the skin. Inhalation or skin penetration must he avoided.

DMSO should never be pipette out using mouth. Always use pipette bulb. The standard chloroanilines and N-1-(naphthyl) ─ ethylenediamine hydrochloride must not be allowed to come into contact with the skin. If they should, then wash the contaminated parts immediately with soap and water thoroughly.

A stock of diluted sodium hypochlorite should be at hand at all times to deal with accidental spillages of chloraniline solution. Spillage on laboratory surface should be treated immediately with the sodium hypochlorite solution, followed by water.

C-3 REAGENTS

C-3.1 Dimethyl Sulphoxide (DMSO) — AR grade.

C-3.2 Hydrochloric Acid — Concentrated (specific gravity -1.18).

C-3.3 Sodium Nitrite — 0.4 percent w/v, analytical grade, freshly prepared (aqueous).

C-3.4 Ammonium Sulphamate — 2 percent *w/v* solution freshly prepared (aqueous).

C-3.5 N-1-(naphthyl) Ethylene — 0.1 percent *w/v* solution diamine hydrochloride freshly prepared (aqueous).

C-3.6 *n*-Butanol — AR grade.

C-3.7 Sand — Acid purified 40-100 micron mesh.

C-3.8 Solvent Mixture

DMSO : 5 volumes

n-Butanol : 2 volumes

Distilled water : 2 volumes

Hydrochloric acid : 1 volume

Mix *n*-butanol, water and HCI. Cool the mixture and add DMSO.

C-3.9 4-Chloroaniline and 3, 4-Dichloroaniline — AR grade.

C-4 APPARATUS

C-4.1 Spectrophotometer — Suitable for use at 554 nm.

C-4.2 Cuvettes — Glass (matched pair) 10 mm.

C-4.3 Water Bath — Thermostatically controlled at 25 °C.

C-4.4 Stop Watch

C-4.5 Standard I.aboratory Glassware

C-4.6 Filter Paper — Whatman No. 541.

C-5 PROCEDURE

C-5.1 Preparation of Calibration Curve

C-5.1.1 Dissolve 0.3498 g of 3, 4-dichloroaniline and 0.2753 g of 4-chloroaniline in solvent mixture (*see* **C-2.8**) in a 250 ml amber volumetric flask. Dilute up to mark with solvent mixture. One ml of stock solution contains 2.5 mg mixed chloroanilines.

C-5.1.2 Dilute this stock solution with solvent mixture as given below:

a) Take 5 ml of stock solution and dilute it to 250 ml with solvent mixture. One ml of this solution contains 50 µg mixed chloroanilines.

b) Take 5 ml of the above solution [*see* **C-5.1.2**(a)] and further dilute to 250 ml with solvent mixture. One ml of this solution contains 1 µg mixed chloroanilines.

Use this solution for preparation of calibration curve.

Transfer using a burette 0, 1, 2, 5, 10, 20, 40 ml into 50 ml amber volumetric flasks.

C-5.1.3 From a burette, add sufficient solvent mixture to make total volume to 40-m1 in each flask. The flasks are incubated in a water bath at 25 °C for 20 min: After exactly 20 minutes, add 2 ml of reagent (*see* **C-3.3**) into each flask and return them to the water bath for exactly 10 min (measure with a stop watch).

Then add 2 ml of reagent (*see* **C-3.4**) into each flask and return them to the water bath for exactly 10 minutes. Swirl the flask occasionally.

Then add 2 ml of reagent (*see* **C-3.5**) into each flask and remove them from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 minutes. Measure absorbance at 554 nm against the blank solution as prepared in **C-5.1.4.**

C-5.1.4 *Preparation of Blank Solution*

Take 40 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25 °C for 20 min. After exactly 20 min, add 2 ml of reagent (*see* **C-3.3**) into the flask and return it to the water bath for exactly 10 minutes. Then add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 min (Swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this blank solution for preparation of calibration curve.

C-5.1.5 Prepare a graph by plotting weight (µg) of chloroanilines contained in each 50 ml flask against absorbance. The linear calibration will pass through the origin/or determine the average absorbance (AA) of 1 µg of mixed chloroanilines by dividing sum of absorbances of all different aliquots of the standard by sum of µg of chloroanilines in all different aliquots of standard.

C-6 DETERMINATION OF CHLOROANILINES

C-6.1 Weigh to the nearest mg, 3.0-3.5 g of finely grated soap and add 10.0-15.0 g of acid purified sand. Transfer the sample and the sand quantitatively into a mortar and grind the mixture thoroughly with a pestle to give a homogenous mass. Transfer the mass to a previously weighed 250 ml flat bottom flask quantitatively and reweigh. Add DMSO (100 ml), stopper firmly and attach the flask to an automatic shaker. Shake for 1 h. Filter the DMSO extract through Whatman No. 541 into a 250 ml amber volumetric flask. Wash the flask and filter paper with small aliquots of DMSO. Allow the filtrate to drain completely, dilute to volume with DMSO and mix. Transfer 20 ml DMSO extract into a 50 ml amber volumetric flask. Add 20 ml of solvent mixture. The flask is incubated in a water bath at 25 °C for 20 min. After exactly 20 min, add 2 ml of reagent (*see* **C-3.3**) into the flask and return it to the water bath for exactly 10 min (measure with a stop watch). Then add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Read the absorbance at 554 nm against blank (prepared as below).

C-6.2 Preparation of Blank

Prepare the blank solution by mixing 20 ml of DMSO extract of sample and 20 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25 °C for 20 min. After exactly 20 min, add 2 ml of distilled water into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 minutes (swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this solution as a blank for reading sample only.

C-6.3 Deduce the amount of chloroanilines (µg) from the calibration graph curve.

NOTE — The determination must be completed in one day.

C-7 CALCULATIONS

C-7.1 Determine the amount of mixed chloroanilines in the aliquot of test solution from the calibration graph.

$$\text{Chloroaniline content (in ppm)} = \frac{250\,(M + M_1)\,M_3}{20\,M_2 M}$$

where,

M = mass in g, of soap,

M_1 = mass in g, of sand.

M_2 = mass in g, of soap and sand transferred to the flask,

M_3 = mass (µg) of mixed chloroanilines found from calibration graph/or

it can be calculated as given below:

$$M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1 µg mixed chloroanilines } (AA)}$$

where

$$AA = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilines in µg}}$$

$$\text{Weight of soap actually used, in } g = \frac{M_2 M}{(M + M_1)}$$

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